PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/GB2004/003247	International filing date *(day/month/year)* 28.07.2004
Priority date *(day/month/year)* 29.07.2003	
International Patent Classification (IPC) or both national classification and IPC G01N1/30, G01N21/64, G01N33/487	
Applicant AMERSHAM BIOSCIENCES UK LMITED	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Michalitsch, R

Telephone No. +49 89 2399-2930

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/003247

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/003247

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☐ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 2,3

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 2,3 are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5-32, 34-36
	No:	Claims	1,4,33
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-36
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/GB2004/003247

Re Item III.

The subject matter of claims 1 to 4, and 33 is unclear insofar as the term "firs time", "first image" and "second time" are merely used as labels. However, these terms must not be used as labels, but relate exclusively to chronological orders or rows. A first time always has to come before a second time. Consequently, claim 2 does in no way restrict the scope of the claim it depends on and claims 2 and 3 are not subject of the provisional examination.

- Furthermore, the meaning of "less capable of identifying" is unclear insofar as it leaves the reader in doubt whether known methods of the prior art fall under the scope of the claim, or not. E.g. all fluorescent markers, which are commonly used in the field, fall under the definition of claim 1, since a fluorescent marker is only capable to identify e.g. a cell nucleus when irradiated with an appropriate wavelength. When the irradiation source is switched off, the dye naturally is less capable of providing this function.
- Furthermore the fluorescence exhibits a time decay etc. Hence such dyes and a broad range of other markers inherently exhibit temporally varying signals as also claimed in claim 5.
- Claim 4 appears to contradict claim 1 insofar as the marker is added only after the recording of the first image. If claim 1 is seen under the light that the marker is not necessarily initially present, this implies that the claim actually is directed towards a standard laboratory method, such as background removal in images. Anyway, such methods belong to the mental furniture and the daily practice of a skilled person and would not even require the citation of relevant prior art in order to state that claimed subject matter lacks an inventive step in the sense of Art. 33(3) PCT.

In conclusion, the claims 1 to 4, and 33 do not fulfill the requirements of Art. 6 PCT. **The definition of the subject matter in the independent claims is so broad that virtually all prior art documents disclosing image subtraction methods appear to anticipate the claimed subject matter or render it obvious.**

Re Item V.

1 The following documents are referred to in this communication:
D1 : US 5 347 139 A (BARKER DAVID L ET AL)

D2 : US 4 389 670 A (CASE ARTHUR L ET AL)
D3 : PATENT ABSTRACTS OF JAPAN vol. 0082, no. 54 (P-315)
&; JP 59 126529 A (FUJI SHASHIN FILM KK)
D4 : DE 100 65 632 A (SMTECH BIOVISION HOLDING AG EC)
D5 : EP 0 401 077 A (BIOLOG VISIONS)

2 INDEPENDENT CLAIM 1

2.1. D2, which is considered to represent the most relevant state of the art, discloses a method for localizing, detecting and quantifying macromolecules, in particular biological materials, with an imaging system (abstract, col.2 lines 50-60) using radioactive or fluorescent markers for generating spacial definitions or providing for background removal etc. (D2, col. 5, lines 6-35).

The difference between the characterising features of D2 and the subject matter of the present application and claims 1 and 33, in particular, consists in the precise definition of the first and second times for taking the images and the use of the same marker in both images (although this reversed in claim 4)

The technical problem to be solved could consequently be seen in the need for minimizing the time/labour effort invested for generating a spatial definition of the biologic entitle.

As already argued above, under the discussion of lack of clarity, the ISA takes the viewpoint that the skilled person is aware of such a procedure, since it comes with his customary practice. As set out before, just the switching off of a light source would provide for the same effect.

Therefore the present independent claims and the claim set as a whole appears to lack an inventive step, in breach of Art. 33(3) PCT.

Related arguments also apply with respect to combinations of D2 with the available prior art, and D5 in particular.

D5 describes methods of removing background and noise from images to generate

dynamic spatial information of labelled objects, relying on the comparison and subtraction of sequentially recorded images (D5, col.4 lines 33-48, col. 18. lines 18-44; col. 19, lines 25-51 as well as claims 16 and 17).

****